

Amendment and Response

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Serial No.: 10/038,984

Confirmation No.: 9705

Filed: January 4, 2002

For: COMPOSITION AND METHOD FOR *IN VIVO* AND *IN VITRO* ATTENUATION OF GENE EXPRESSION
USING DOUBLE STRANDED RNA

Remarks

The Office Action mailed December 29, 2008 has been received and reviewed. Claims 75 and 90 having been amended, claim 84 having been canceled, without prejudice, and claims 99-120 having been added, the pending claims are claims 75-76, 78-79, 82-83, and 85-120. In addition to the remarks made in the reply dated October 14, 2008, the Examiner is requested to consider the following remarks. Reconsideration and withdrawal of the rejections are respectfully requested.

Support for the amendment of claim 75 may be found in the specification at, for example, page 34, line 14 through page 35, line 4.

Support for the amendment of claim 90 may be found in the specification at, for example, page 12, lines 1-15.

Support for new claim 99 may be found throughout the specification including, for example, page 2, lines 25-27, and page 34, line 14 through page 35, line 4.

New claims 100-117 recite the subject matter of claims 78-79, 82-83, and 85-98, respectively.

Support for new claim 118 may be found in the specification at, for example, the examples and page 4, lines 19-23.

Support for new claim 119 may be found in the specification at, for example, page 10, lines 17-25.

Support for new claim 120 may be found in the specification at, for example, page 10, line 26 through page 11, line 7.

The 35 U.S.C. §112, First Paragraph, Rejection

The Examiner has rejected claims 75, 76, 78, 79, and 82-98 under 35 U.S.C. §112, first paragraph, as not complying with the enablement requirement. Specifically, the Examiner asserts "the working examples of the specification cannot be extrapolated to the full scope of the claims, which is any vertebrate cell" (Office Action at page 12). This rejection is respectfully traversed.

Independent claim 75 has been amended to indicate that dsRNA is added in an amount that does not induce interferon- α/β mediated toxicity.

The Examiner acknowledges that the specification is enabling for attenuating the expression of the disclosed target genes in the disclosed species. Applicants believe that the examples present evidence of the claimed invention using a sufficient number of genes and species of vertebrate cells in order to support the claimed genus. The standard is set out in M.P.E.P. § 2164.02, where it states that

For a claimed genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art (in view of level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation. Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation.

Applicants have demonstrated, by working, representative examples, the specific attenuation of gene expression using dsRNA in zebrafish embryos (Example I), chick neural crest tissue (Example II), and rat ROS cells (Example III). In zebrafish embryos the targeted genes were GFP, T gene, Pax6.1, Nkx 2-7, and both T gene and Pax6.1 simultaneously. The HirA gene was targeted in chick neural crest tissue, and a plasmid based GFP gene was targeted in murine (rat ROS) cells¹. It can be seen that the Applicants have shown successful gene silencing using dsRNA in a wide range of systems targeting a wide range of genes, and have provided numerous working examples. It is submitted that the Applicants have made an adequate showing of enablement in the specification for the claimed genus.

The reasons given by the Examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation are (i) the failure of the specification to

¹ As discussed in the Preliminary Amendment submitted April 28, 2003, rat ROS cells were used in Example III, not NIH 3T3 cells.

teach how to overcome non-specific effects, and (ii) the unpredictability based on the state of the prior art. As detailed below, the Applicants submit the Examiner's reasons for lack of enablement are not adequate to establish that a person skilled in the art could not use the genus as a whole without undue experimentation.

The Examiner asserts there is no disclosure in the specification on how to overcome the non-specific inhibition of gene expression as reported in the prior art when dsRNAs are added to vertebrate cells. The Applicants disagree, and request consideration of the disclosure at page 34, lines 26-29, of the specification which states "the amount of double-stranded RNA that was used to generate the phenotypes is much less than is necessary to cause [] interferon-mediated cell toxicity." The specification also discloses that approximately 10^6 molecules of dsRNA were injected into zebrafish embryos (specification at page 22, lines 11-17), and ranged from 1.6×10^5 molecules to 5×10^8 molecules (specification at page 27, lines 9-21). The inhibition of phenotypes was specific to the targeted genes, and injection of control dsRNA at the same concentrations did not cause a detectable deviation from wild type expression levels or phenotype (specification at page 35, lines 2-4). Likewise, in Example II explanted neural chick tissue was exposed to approximately 10^6 dsRNA molecules per nanoliter (specification at page 35, lines 24-26), and the inhibitory activity was specific to the targeted gene (specification at page 36, lines 3-4). Thus, the specification provides direction and guidance on how to avoid interferon-mediated cell toxicity in a cell when dsRNA is introduced.

The Examiner believes the results of the working examples are contradictory to what the skilled person would expect, and cites Oates et al. (Developmental Biology, 2000) and Zhao et al. (Developmental Biology, 2001). Both of these cited documents show that non-specific inhibition of gene expression is dependent on the amount of dsRNA. Oates et al. report that dsRNA at doses of approximately 0.005 picograms (5×10^5 molecules) did not alter phenotypes of embryos, but dsRNA at doses of 40 picograms per embryo resulted in non-specific inhibition of gene expression (Oates et al., page 25, left column). Zhao et al. report that injection of 1.5

picograms dsRNA/embryo resulted in normal development of nearly all embryos, while injection of greater amounts of dsRNA resulting in increasing numbers of abnormal embryos (Zhao et al., Figure 4). Thus, Oates et al. and Zhao et al. support the insight presented in the present application that a non-specific response to dsRNA is dependent upon the amount of dsRNA supplied to a cell.

The Examiner notes that the working examples use dsRNAs with sizes between 141 to 298 base pairs to specifically inhibit target gene expression, but asserts that the skilled person would nonetheless expect long dsRNAs to non-specifically inhibit gene expression. This assertion is supported by citing Elbashir et al. (Nature, 2001, of record), who state that dsRNA >30bp in the cytoplasm of mammalian cells activates the protein kinase PKR and stalls translation. There is no statement in Elbashir et al. regarding the state of the art that suggests dsRNA can be supplied to a cell in an amount that will not induce interferon- α/β mediated toxicity. Thus, the statement in Elbashir et al. regarding the length of dsRNA and non-specific inhibition does not suggest claim 75 as amended is unpredictable.

The Examiner also states that it is critical to use suitable models to predictably extrapolate experimental results to other cell types, but the results observed in the working examples are not broadly applicable to all vertebrate cells (Office Action at page 6, last paragraph). In addition to using vertebrate cells as diverse as fish cells, mammalian cells, and avian cells, the Applicants have used an organism that is designed to be representative of the genus. The zebrafish is not merely a fish but is rather a model organism for studies of vertebrate organisms. In fact, experts have promoted the use of the zebrafish as a model for human biology. See "The Zebrafish Model Organism Database" available at http://zfin.org/zf_info/dbase/db.html.

Wianny et al. (Nature Cell Biology, 2000) is cited by the Examiner to further support the assertion that the results observed in the examples are not broadly applicable to all vertebrate cells. Wianny et al. state that "it is possible that the early mouse embryo is incapable of an

interferon response and that there may still be difficulties in using RNAi at later stages" (p. 73, under Discussion). Despite the pessimism expressed by Wianny et al., Applicants have demonstrated that it is possible to achieve target specific regulation of gene expression in vertebrate cells by administering a double stranded RNA corresponding to the target gene. The skilled artisan could clearly apply that invention to other cell types of other vertebrate species given Applicants' data absent undue experimentation. In fact, Wianny et al. supports the surprising nature of Applicants' invention and is evidence that the claimed invention would not have been evident to the skilled artisan until they were presented with Applicants' data.

The Examiner asserts that none of the working examples describe *ex vivo* treatment of a cell followed by implantation into any organism for any purpose (Office Action at page 4). The Examiner is requested to consider the disclosure of U.S. Provisional Patent Application 60/117,635 (the '635 provisional application). The '635 provisional application was incorporated by reference in the present application at the time of filing. The '635 provisional application exemplifies *ex vivo* treatment of chick neural crest tissue with dsRNA and re-implantation of the treated explant into embryos (see page 2 and Figures 7 and 8). Briefly, cardiac neural crest explants were treated with dsRNA specific for chick HIRA *in vitro*, implanted back into embryos after treatment, and the embryos were allowed to develop to embryonic day 8. Embryos in which the cardiac neural crest had been treated with the HIRA dsRNA manifested a significant increase in persistent truncus arteriosus (a heart defect) at day 8. Thus, the specification clearly enables an *ex vivo* method that includes treatment of an explanted cell with dsRNA followed by implantation into an organism. Moreover, this working example of successful *ex vivo* treatment followed implantation is evidence that, in view of the teachings of the present specification, undue experimentation is not required to practice an *ex vivo* method to treat a disease or pathogen.

In summary, the Applicants note that the presumption is that an application is enabled, and that this is overcome only if the Examiner can show that undue experimentation is necessary

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to use the invention as claimed. Furthermore, the mere fact that experimentation may be involved, and even be complex, does not necessarily make the experimentation undue. Reconsideration and withdrawal of the rejection of the pending claims under 35 U.S.C. §112, first paragraph, is accordingly requested.

The 35 U.S.C. §102 Rejection

The Examiner has also rejected claims 75, 76, 78, 79, 82-91 and 93-98 under 35 U.S.C. §102(e) as being anticipated by Fire et al. (U.S. Patent 6,506,559). This rejection is respectfully traversed.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." M.P.E.P. §2131. Fire et al. notes that some organisms have a dsRNA dependent protein kinase that activates a panic response (Fire et al., col. 16, lines 7-22). The only teaching on how to avoid a panic response appears to be the suggestion that RNA structure can be modified (Fire et al., col. 7, lines 30-41). In contrast, independent claim 75 has been amended to recite supplying the vertebrate cell with double stranded RNA in an amount sufficient to "not induce interferon- α/β mediated toxicity." Fire et al. do not teach or suggest supplying any cell, much less a vertebrate cell, with double stranded RNA in an amount that will not induce interferon- α/β mediated toxicity. Since Fire et al. do not teach or suggest this, Fire et al. cannot anticipate claims 75, 76, 78, 79, 82-91 and 93-98.

For at least this reason, reconsideration and withdrawal of the present rejection is respectfully requested.

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The Examiner has rejected claims 75, 76, 78, 79, and 82-98 under 35 U.S.C. §103(a) as being unpatentable over Fire et al., in view of Ekenberg et al. (Promega Notes Magazine Number 46, 1994, pages 14-17). This rejection is respectfully traversed.

Independent claim 75 recites supplying the vertebrate cell with double stranded RNA in an amount sufficient to "not induce interferon- α/β mediated toxicity." The disclosure of Fire et al. does not teach or suggest supplying any cell, much less a vertebrate cell, with double stranded RNA in an amount that will not induce interferon- α/β mediated toxicity. Ekenberg et al. likewise does not teach or suggest supplying a cell with double stranded RNA in an amount that will not induce interferon- α/β mediated toxicity. Thus, Ekenberg et al. does not supplement the deficiencies of Fire et al. Since Ekenberg et al. does not supplement the deficiencies of Fire et al., the cited documents do not teach or suggest all the claim limitations. Thus, the Examiner has failed to establish a *prima facie* case of obviousness for claims 75, 76, 78, 79, and 82-98.

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Summary

It is respectfully submitted that the pending claims are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives at the telephone number listed below if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted

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CERTIFICATE UNDER 37 CFR §1.6:

The undersigned hereby certifies that this paper is being transmitted via the U.S. Patent and Trademark Office electronic filing system in accordance with 37 CFR §1.6(a)(4) to the Patent and Trademark Office addressed to the Commissioner for Patents, Mail Stop Amendment, P.O. Box 1450, Alexandria, VA 22313-1450, on this 29 day of April, 2009.

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